yellow light is a striking feature for each of the objects mentioned. Quantitative measures of intensity have not yet been made, but there is every reason to believe that the nebular condensations will reveal negative color indices of large amount. The knots of nebulosity are certainly bluer than the bluest of the neighboring stars, and one is reminded of the great photographic activity of the central star in the Ring Nebula in Lyra.

Whatever the spectral character of these outlying regions may prove eventually to be, it must differ from that of the central nucleus, for the three central nuclei and the secondary nucleus at the end of one of the branches of M 51 are all much stronger in yellow light than in blue. Here the color seems to be in accordance with the typical absorption spectrum found in all similar objects thus far observed.

It is still too early for any general conclusion, but preliminary photographs of other spirals suggest similar results; and it seems not unlikely that the phenomena described are typical of this class of objects.

In contrast to the spirals it is of interest to note the results for the bright planetary N.G.C. 3242, which is also illustrated. In this instance no important differences are revealed by the blue and yellow exposures, at least none which cannot be accounted for by possible differences in gradation on the two kinds of plates.

[1] Newcomb-Engelmann, *Populäre Astronomie*, Fünfte Auflage, p. 672, Leipzig, 1914.
[2] These PROCEEDINGS, **1**, 590 (1915).
[3] An isochromatic plate exposed behind a yellow filter.
[4] *Astroph. J.*, **9**, 133 (1899).
[5] *Ibid.*, **21**, 389 (1905).
[6] An unpublished result obtained in 1909.

THE ACTION OF ALKALI IN THE PRODUCTION OF LIPOLYTICALLY ACTIVE PROTEIN

By K. George Falk

HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK

Received by the Academy, August 22, 1916

Introduction.—A summary of an extended experimental study of the lipolytic or ester-hydrolyzing enzymes was presented in these PROCEEDINGS last year.[1] The changes in the lipases themselves under various conditions were the main objects of the investigation at that time, as a preliminary to the possible elucidation of the chemical structure of the active groupings. The investigation of the factors which control the loss or destruction of this enzymatic hydrolyzing activity

appeared to offer the most promising field for further study; a preliminary report on the results of this study is here presented.

The materials used were *Esterase preparation*, a clear, dialyzed and filtered water-extract of oil-free and husk-free castor beans 0.5 g. to 60 cc.; and *lipase preparation*, a mixture from the 1.5 normal NaCl solution extract of water-extracted castor beans, 1.0 g. to 100 cc., dialyzed until salt-free. The former, a clear colorless solution, was more suitable for following the changes under different conditions than the latter, which was a suspension of globulin in water.

The activity tests were carried out with 1 cc. ethyl butyrate or 0.5 cc. glyceryl triacetate at 38° for 24 or 48 hours, and the results given as the number of cubic centimeters of 0.1 normal alkali required for neutralyzing the acid produced with phenolphthalein as indicator, with suitable corrections for blanks.

Inactivation of the enzymes by acid.—The hydrogen-ion concentrations were determined by color comparison with the standard solutions and indicators recommended by A. A. Noyes.[2] The esterase preparation, $H^+ = 10^{-7.0}$, brought to $H^+ = 10^{-3.5}$ with acetic acid for 24 hours and then back to $H^+ = 10^{-7.0}$, lost its activity completely. At $H^+ = 10^{-4.5}$ under the same conditions for the same length of time, one-third of the activity was lost, while at $H^+ = 10^{-6.0}$ about one-eighth was lost. The difficulty of color comparison with the lipase preparation made difficult an exact determination of the H^+ ion concentration at which inactivation took place. A larger H^+ ion concentration than with the esterase preparation appeared to be necessary to produce a corresponding effect.

Inactivation by alkali.—The esterase preparation lost one-eighth of its activity after being kept at $H^+ = 10^{-8.0}$ for 24 hours, and became practically inactive at $H^+ = 10^{-10.5}$ and $10^{-11.0}$. The lipase preparation dissolved to form a cloudy solution at $H^+ = 10^{-11.0}$. Kept at this for 18 hours and then brought back to $H^+ = 10^{-7.0}$, one-half of the activity was lost. At about $H^+ = 10^{-12}$, one-third of the activity remained, and at about $H^+ = 2 \times 10^{-12}$, one-sixth remained.

Inactivation by alcohols and by acetone.—It was shown previously that dilute solutions of methyl alcohol, ethyl alcohol, and acetone inhibited the action of both preparations. Solid preparations made by precipitation and washing with alcohol were always inactive. Solid esterase preparations, precipitated and washed with acetone, were active in a number of cases; but the activity was much smaller than that of the corresponding solutions from which they were prepared. Similar solid lipase preparations were always inactive.

Inactivation by salts.—The action of salts on these enzymes has already been described. Some produced marked inactivation, others less, while some accelerated the hydrolytic actions.

Inactivation by heat.—Like all enzymes, the esterase and lipase are both inactivated by heating their aqueous solutions or suspensions for a few minutes at 100°. The original oil-free and husk-free castor beans, on being heated dry at 100–110° lost 50–80% of their lipolytic activity; the same loss of weight in a vacuum desiccator over phosphorus pentoxide was not accompanied by loss in activity. Drying first, and then heating (the latter causing only 0.1–0.2% greater loss in weight) produced 50–80% loss in activity.

Nature of the chemical changes involved in the inactivations.—The summary of the different ways in which the esterase and lipase preparations may be inactivated makes it appear at first sight as if different reactions occurred in the inactivations. If, however, a definite chemical group is responsible for a definite enzyme action, it might perhaps be more reasonable to assume that inactivation followed a definite reaction. The preparations were essentially protein in character. There is no evidence that a dehydration, or loss of the elements of water, causes the inactivation. Some of the reactions indicate that a possible hydrolysis may be a cause of inactivation. With proteins, hydrolysis is generally taken to occur with the – CO–NH – group, which goes over into the – COOH NH_2 – groups. Experiments with all the inactivations showed in no case an increase in the formal titration as would be expected in such a reaction, and therefore makes the assumption of such a hydrolysis improbable. Coagulation of the material accompanied some of the inactivations. This physical change alone does not appear satisfactory as an explanation, some change in chemical structure unquestionably accompanying or producing the physical phenomenon. Furthermore, the lipase material in suspension in water showed the same activity as in 1.5 normal sodium chloride solution when tested immediately.

The explanations of the chemical changes accompanying inactivation so far suggested are not satisfactory. The reagents used are simple. It is difficult to conceive of a very deep-seated chemical reaction taking place under so many different conditions, none of a complex nature. To the writer the only chemical change which appears probable under these conditions is that involving a simple rearrangement within the molecule, such as a tautomeric change involving the change in position of a hydrogen atom. In considering the structure

of proteins, it is evident that such a rearrangement is possible in the peptide linking.

The hypothesis to be suggested is that the active grouping of the esterase and lipase preparations is of the enol structure $-C(OH)=N-$, the specific actions being dependent in part upon the groups combined with the C and N, and that inactivation consists primarily in a rearrangement to the keto group $-CO-NH-$. Such structures have been proposed at different times as indicating the difference between proteins in living matter, and proteins not in living matter.

Since strong alkali, as a rule, favors the formation of the enol structure in such tautomeric changes, a way is open to test the hypothesis.

Activation of proteins by alkali.—One gram of inactive solid lipase-preparation, washed and dried by means of alcohol, was allowed to stand 24 hours with 25 cc. 10% NaOH solution (and toluene). The brown suspension or mixture was diluted with 100 cc. water and dialyzed against running water for 24 hours to remove the greater part of the alkali. The volume increased to 410 cc. The mixture was brought to a very faint pink color toward phenolphthalein with hydrochloric acid, and the hydrolytic action of 50 cc. portions (corresponding to 0.12 g. original material) was tested for 48 hours. An action (corrected for blanks) of 0.24 cc. was found with ethyl butyrate, and 0.73 cc. with glyceryl triacetate.

Many experiments were also carried out with casein (Kahlbaum's preparation "nach Hammarsten"), of which the following may be cited. Two grams of casein were mixed with 25 cc. 1 normal NaOH solution (and toluene). After 24 hours at room temperature, a grayish-brown and dialyzed 48 hours, the volume increasing to 280 cc.; the H^+ ion concentration was then brought to $10^{-7.0}$, and the hydrolytic actions of 40 cc. portions, corresponding to 0.3 g. of casein each, were tested for 48 hours. The actions found were 0.08 cc. with ethyl butyrate and 0.48 cc. with glyceryl triacetate. A large number of similar experiments were carried out in which the alkali was removed either by dialysis alone or by direct neutralization with acid. There was marked action on glyceryl triacetate, but only very slight action on ethyl butyrate.

The strength of the alkali, between 0.1 normal and 3 normal, used in the preliminary treatment, appeared to have small influence on the activity produced; but great influence was exerted by the H^+ ion concentration of the solutions in the activity tests. This last effect may be shown by a series of results with 1 normal NaOH solution in which

the alkali was neutralized directly to different points. Twenty-four hours' action on glyceryl triacetate gave the following results:

H^+ ion concentrations	10^{-4}	10^{-6}	10^{-8}	10^{-10}
Activities	0.15	0.10	1.17	1.47

Two similar experiments gave the following results:

H^+ ion concentrations	10^{-7}	10^{-8}
Activities	0.91	1.68

The action was therefore greater in slightly alkaline solution.

[1] These PROCEEDINGS, **1**, 136 (1915).
[2] *J. Amer. Chem. Soc.* (1911).

THE EXCRETION OF ACIDS BY ROOTS

By A. R. Haas

LABORATORY OF PLANT PHYSIOLOGY, HARVARD UNIVERSITY

Received by the Academy, August 28, 1916

Whether roots excrete acid, other than carbonic, has long been a matter of controversy. The problem is important not only because acids dissolve plant food from the soil, but also because it involves the fundamental questions of the reaction of protoplasm and of the mechanism of secretion.

The problem has been greatly complicated by the failure of many investigators to distinguish between the effects of dead and of living cells.

Becquerel early pointed out that all seedling roots when laid on moist neutral litmus paper possess the property of giving it a lasting red color, which he believed was due to excretion of acetic acid. Boussingault thought that the acid might be lactic, while other investigators left the nature of the acid undetermined. The alkali salts of formic acid have been reported by Czapek[1] in the culture solution of *Lepidium* and *Hordeum* seedlings. The formic acid was considered as coming not from the root hairs but from the sloughing off of root cap cells and their secondary decomposition. The drops occurring on root hairs in a moist atmosphere were found to give no acid reaction. The solvent[2] action of plant roots has been considered to be due not alone to the respired CO_2 but also to organic acids because phosphorite (which can be taken up to some degree by plant roots), requires a strong acid to dissolve it. Kunze[3] believes that there is no free mineral acid in the root excretion of higher plants, but that the acid reaction is due to